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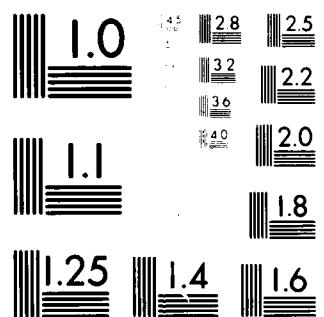
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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)		
REPORT DOCUMENTATION PAGE		
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
	AD-A085 937	(2)
4. TITLE (and Subtitle)	Comparison of Some Physical and Immunoologic Properties of a Phase I and a Phase II Q Fever Vaccine in Guinea Pigs	
5. AUTHOR(s)	J.W. Johnson, R.F. Wachter, G.A. Higbee, C.E. Pedersen, Jr.	
6. CONTRACT OR GRANT NUMBER(s)		
7. PERFORMING ORGANIZATION NAME AND ADDRESS	U.S. Army Medical Research Institute of Infectious Disease, Aerobiology Division (SGRD-VIA-E) Fort Detrick, Frederick, Maryland 21701	
8. CONTROLLING OFFICE NAME AND ADDRESS	U.S. Army Medical Research and Development Command Office of the Surgeon General, Department of the Army, Frederick, MD 21701	
9. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	(12) 14 LEVEL	
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	(16) 3M762776A1842 A841-00-053	
11. REPORT DATE	(11) 9 May 80	
12. NUMBER OF PAGES	13	
13. SECURITY CLASS. (of this report)	UNCLASSIFIED	
14. DECLASSIFICATION/DOWNGRADING SCHEDULE		
15. DISTRIBUTION STATEMENT (of this Report)	Cleared for public release; distribution unlimited (25 March 1980)	
16. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)	(17) JUN 26 1980 CTE	
17. SUPPLEMENTARY NOTES	Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in the Journal of Infectious Diseases	
18. KEY WORDS (Continue on reverse side if necessary and identify by block number)	GUINEA PIGS, IMMUNOLOGY, Q FEVER, PHASE I, PHASE II, VACCINES <u>C. burnetii</u>	
19. ABSTRACT (Continue on reverse side if necessary and identify by block number)	A highly purified phase I Q fever vaccine was evaluated by comparing its physical, serologic and protective properties with those of a phase II vaccine currently in use. The concentration of rickettsiae was approximately the same in both preparations. The phase I vaccine had a median protective dose in guinea pigs of 1.8 µg, compared with 27.3 µg needed for the phase II vaccine. Median doses required for detectable serum complement-fixing, microagglutinating and immunofluorescing antibodies were also lower by factors of 1.5 to 20 times for the phase I vaccine.	

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Comparison of Some Physical and Immunologic Properties of a Phase I and  
a Phase II Q Fever Vaccine in Guinea Pigs

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Running head: Comparison of Q Fever Vaccines

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

The authors are indebted to Ms Marilyn J. England, Mr. William J. Ruffa and Mr. Gilbert P. Briggs for excellent technical assistance throughout this study.

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(25 March 1980)

## ABSTRACT

A highly purified phase I Q fever vaccine was evaluated by comparing its physical, serologic and protective properties with those of a phase II vaccine currently in use. The concentration of rickettsiae was approximately the same in both preparations. The phase I vaccine had a median protective dose in guinea pigs of 1.8 µg, compared with 27.3 µg needed for the phase II vaccine. Median doses required for detectable serum complement-fixing, microagglutinating and immunofluorescing antibodies were also lower by factors of 1.5 to 20 times for the phase I vaccine.

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In 1960, a formalinized Q fever vaccine was prepared by Berman et al. [1] from the Henzerling strain of Coxiella burnetii. This experimental vaccine, derived from a predominantly phase II culture, is still being used for the immunization of laboratory workers and others exposed to this organism. While phase II vaccines usually provide good protection, they are difficult to prepare [2], and require considerable effort and time for the removal of contaminating host material [3]. In addition, the organisms often agglutinate spontaneously when purified. The incidence and severity of side reactions, especially among previously exposed or vaccinated individuals, also detracts from their acceptability as immunizing agents [4].

Ormsbee et al. [2], in 1963, demonstrated that phase I strains of C. burnetii could be separated from infected yolk sac suspensions in a relatively pure state and showed little tendency to agglutinate spontaneously or to retain yolk components. They reported that in guinea pigs, vaccines prepared from these purified rickettsiae were effective immunogens, possessing significantly lower dose requirements and lower reactogenicities than similar preparations made from phase II strains.

In 1970, Spicer et al. [5] published a method for the purification of phase I C. burnetii from infected yolk sacs that involved extraction with Freon 113<sup>R</sup> and passage through a brushite column to remove contaminating host material. Their data indicated that rickettsial suspensions produced by this procedure were free of detectable host material. Using this method Spicer and DeSanctis [6] prepared five large lots of a phase I antigen suitable for vaccine use. The present communication reports an investigation of some physical properties of this experimental vaccine and the protective and serologic responses it

elicited in guinea pigs. These data are compared with similar characteristics of the phase II vaccine prepared by Berman et al. [1].

#### Materials and Methods

Vaccines. The phase II vaccine was prepared in 1960 by Berman et al. [1] at the Walter Reed Army Institute of Research from the 22nd egg passage of the Henzerling strain of C. burnetii. Lot #7 was used in this study.

The phase I vaccine was prepared by Spicer and DeSanctis [6] from the third egg passage of the Henzerling strain. Lot #4 of this vaccine was used in these experiments.

Rickettsial strain. The third egg passage of a phase I Henzerling strain of C. burnetii [7] was used to challenge vaccinated guinea pigs. The challenge dose was  $1 \times 10^5$  median egg infective doses ( $EID_{50}$ ) of the organism. Rickettsial suspensions were assayed in 6-day-old embryonated eggs (Spafas, Norwich, Conn.) as described by Robinson and Hasty [7].

Animals. Female, Hartley strain guinea pigs weighing 300-400 g were caged in groups of ten, given commercial guinea pig pellets and water ad libitum; the diet was supplemented with fresh kale. About 10% of the guinea pigs from each experiment were bled by cardiac puncture prior to vaccination and their sera were tested for Q fever antibodies.

Serology. Humoral antibody was assayed by the microagglutination (MA) test of Fiset et al. [8], the complement fixation (CF) test of Casey [9], and the immunofluorescent antibody assay (IFA) procedure of Bozeman and Elisberg [10]. Antigens were prepared by the method of Ormsbee et al. [2] from infected yolk sacs of the fourth egg passage of a phase I Henzerling strain of C. burnetii. Phase II antigen was derived

from purified phase I by extracting the rickettsiae with trichloroacetic acid [8] to remove the phase I component.

Physical properties. Buoyant densities were determined in CsCl gradients centrifuged at 105,000 g for 24 hr as described by Wachter et al. [11]. Direct counts were made by the method of Silberman and Fiset [12]. Optical density (O.D.) of each vaccine was measured at 420 nm, after exhaustive dialysis against distilled water, according to the procedure of Fiset et al. [8]. A Beckman DB-1 prism spectrophotometer was used for O.D. measurements, employing the instrument's fixed medium-slit width and a cuvette having a path length of 1.0 cm. The weight of antigen per milliliter of reconstituted phase I vaccine was obtained from the data of Spicer and DeSanctis [6]. For the phase II vaccine the weight was calculated by multiplying the phase I vaccine weight by a ratio of the O.D. of the two preparations.

Experimental design. Groups of ten guinea pigs were vaccinated sc with 0.5-ml dose of varying dilutions of each vaccine. In some experiments, half the vaccine dose was injected on day 0 and the remainder on day 7. On the 20th day after the initial dose, animals were bled by cardiac puncture and on the following day were challenged ip with 1.0 ml of a live virulent C. burnetii suspension. The temperature of each guinea pig was recorded daily for 10 days following challenge and the number of fever days  $\geq$  40 C for each group was used to calculate median protective doses by probit analysis [13]. Median serologic doses for each vaccine were calculated by the Reed-Muench method [14] using the number of guinea pigs in each group having a titer  $\geq$  1:8 in the MA and CF tests or  $\geq$  1:10 in the IFA test.

In each experiment, a group of ten guinea pigs was inoculated with saline and the temperature of each animal recorded daily for 10 days prior

to challenge. These animals provided a temperature baseline for the experiment and, following challenge, served as unvaccinated controls.

### Results

Some physical properties of the two vaccines are shown in Table 1. When properly reconstituted, both had about the same number of organisms per milliliter, but differed noticeably in O.D. The ratio of these O.D. measurements indicated that the phase II vaccine contained about 36% more dispersed solids than the phase I preparation. The O.D. of dilutions of each vaccine, when plotted against antigen concentration, gave straight lines which, on extrapolation, appeared to pass through the origin.

Centrifugation of the phase I vaccine in CsCl solution produced a single band at a density of 1.32 g/ml, the density reported by Hoyer et al. [14] for phase I C. burnetii. No other bands were found. The phase II vaccine also formed a band at this density level as well as a diffuse particulate band centered at 1.22 g/ml, the density reported for phase II organisms. Visually, the lower band of the phase II vaccine was easily observable, and smears indicated that a substantial number of organisms were present at the phase I density level.

Table 2 shows the micrograms of vaccine needed to elicit a median protective or serologic response in vaccinated guinea pigs. A comparison of the relative efficacies of the two vaccines can best be made by reference to the single-dose columns of the table. The largest dose used with the phase II vaccine, 30 µg, did not cause detectable antibody to develop in any of the animals tested; however, several guinea pigs were protected at this level and a median protective dose

could be calculated. A median protective dose of 1.8  $\mu\text{g}$  was found for the phase I vaccine. Median dose estimates were also obtained for phase I and phase II MA and phase II IFA. Median protective doses for the two vaccines were significantly different at the  $P = 0.001$  level.

Using a divided dose schedule, with half doses given on days 0 and 7, an increase in the efficacy of the phase I vaccine could be shown as is indicated in the 1- and 2-dose columns for this vaccine in Table 2. About a five-fold reduction in the median protective dose was obtained with this schedule. Median protective doses for the two phase I vaccine regimens were significantly different at the  $P = 0.01$  level. Similar or greater reductions in median serologic doses were also observed. Only the phase I CF antibody response remained undetectable at 30  $\mu\text{g}$ , the highest dose used for this vaccine.

A breakthrough challenge dose for the phase I vaccine was also measured in vaccinated guinea pigs. A group of animals was inoculated with two vaccine doses of 15  $\mu\text{g}$  each, given 7 days apart. On day 21 postinoculation, groups of six animals each were challenged ip with live phase I C. burnetii using doses varying from  $1 \times 10^1$  to  $1 \times 10^8 \text{ EID}_{50}$ . From febrile responses of these animals, the breakthrough dose was calculated to be  $3.1 \times 10^7 \text{ EID}_{50}$ .

#### Discussion

From the data reported here (Table 2), it would appear that the phase I antigen of Spicer and DeSanctis [6] is a better immunizing agent than the phase II vaccine. Not only was the protective dose found to be lower in guinea pigs, but the serologic data indicated that MA, CF and IFA phase II and MA and IFA phase I responses also developed. This

broad immunologic pattern can be helpful in a vaccination program where the protective antigen is not readily detectable by serologic methods, since it provides evidence that the recipient is responding to the vaccine even though the antigens detected may not necessarily be related to protection.

Studies of the physical properties of the phase I vaccine indicated that it contained considerably less extraneous material than the phase II preparation. This was shown by comparing O.D. measurements of the vaccines when each had been properly reconstituted. Since both preparations contained about the same number of organisms at these concentrations, and appeared equally well dispersed, the difference suggests the presence of more nonrickettsial material in the phase II vaccine.

The serologic studies of the phase II vaccine reported here gave no indication of the presence of a phase I component. Other studies [4] also have indicated that it contained only small amounts of this antigen. However, in CsCl gradients, an easily observable band developed at the phase I density level and was found to contain substantial numbers of organisms. Apparently, few of these organisms were in phase I and this band probably was composed at least in part of phase II organisms whose densities had been altered by removal of buoyant host material. Such density changes have been reported by Urvölgyi and Schramek [16] to occur with phase II strains during purification. Also, Canonico et al. (17) and Wachter et al. (11) using sucrose gradients have demonstrated that several morphologically distinct cell types with different densities are present in this phase II Q fever vaccine. A combination of these mechanisms as well as the presence of dense extraneous material in this vaccine could account for a band at the phase I density level.

All guinea pigs in this study were challenged with a phase I strain of C. burnetii; the effects of a phase II challenge were not determined. Some phase II challenge studies have been reported by Ormsbee et al. [2], who showed that on a weight basis, vaccination of guinea pigs with antigens of either phase was equally effective against a phase II challenge, but that lower doses of phase I vaccines could be used to protect against phase I challenge. Since naturally acquired Q fever infections are usually caused by phase I organisms, a phase I antigen would appear to be the more useful vaccine. The lower dose requirements should reduce adverse reactions. It is always possible to increase the immunizing dose if protection against phase II infections seems desirable.

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Table 1. Physical properties of phase I and phase II Q fever vaccines.

Property	Vaccine	
	Phase I	Phase II
Organisms/ml (direct count)	$1.6 \times 10^9$	$1.5 \times 10^9$
Buoyant density in CsCl (g/ml)	-*	1.22
	1.32	1.32
Optical density†	0.27	0.42

\* No band observed at 1.22 g/ml.

† Reconstituted vaccine dialyzed against distilled water.

Table 2. Median protective and serologic doses of experimental phase I and phase II Q fever vaccines in guinea pigs.\*

Test antigen	Median dose ( $\mu\text{g}$ )		
	Phase II vaccine		Phase I vaccine
	1 dose	1 dose	2 doses <sup>†</sup>
Protection ( $\text{PD}_{50}$ )	27.3 (4.2-176) <sup>‡</sup>	1.8 (0.50-5.7) <sup>‡</sup>	0.32 (0.1-1.0) <sup>‡</sup>
<b>Phase I serology</b>			
Complement fixation ( $\text{CF}_{50}$ )	> 30	> 30	> 30
Microagglutination ( $\text{MA}_{50}$ )	> 30	19	4.0
Immunofluorescent assay ( $\text{IFA}_{50}$ )	> 30	> 30	5.3
<b>Phase II serology</b>			
Complement fixation ( $\text{CF}_{50}$ )	> 30	> 30	0.59
Microagglutination ( $\text{MA}_{50}$ )	> 30	7.3	0.32
Immunofluorescent assay ( $\text{IFA}_{50}$ )	> 30	1.4	0.22

\* Animals were bled 20 days after initial vaccine dose and challenged the following day with  $1 \times 10^5 \text{ EID}_{50}$  of phase I C. burnetii.

<sup>†</sup> One half dose given on day 0 and 7.

<sup>‡</sup> 95% confidence interval.